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(54) Title: A NOVEL HUMAN CHECKPOINT KINASE, hCDS1, COMPOSITIONS AND METHODS <p style="text-align: center;">Predicted hCDS1 Amino acid sequence</p> <pre> 1 MSRESDV E A Q Q S H G S S A C S Q P H G S V T Q S Q G S S S Q S Q G I S S 40 41 S S T S T M P N S S Q S S H S S S G T L S S L E T V S T Q E L Y S I P E D Q E P 80 81 E D Q E P E E P T P A P W A R L W A L Q D G F A N L E C V N D N Y W F G R D K S 120 121 C E Y C F D E P L L K R T D K Y R T Y S K K H F R I F R E V G P K N S Y I A Y I 160 161 E D H S G N G T F V N T E L V G K G K R R P L N N N S E I A L S L S R N K V F V 200 201 F F D L T V D D Q S V Y P K A L R D E Y I M S K T L G S G A C G E V K L A F E R 240 241 K T C K K V A I K I I S K R K F A I G S A R E A D P A L N V E T E I E I L K K L 280 281 N H P C I I K I K N F F D A E D Y Y I V L E L M E G G E L F D K V V G N K R L K 320 321 E A T C K L Y F Y Q M L L A V Q Y L H E N G I I H R D L K P E N V L L S S Q E E 360 361 D C L I K I T D F G H S K I L G E T S L M R T L C G T P T Y L A P E V L V S V G 400 401 T A G Y N R A V D C W S L G V I L F I C L S G Y P P F S E H R T Q V S L K D Q I 440 441 T S G K Y N F I P E V W A E V S E K A L D L V K K L L V D D P K A R F T T E E A 480 481 L R H P W L Q D E D M K R K F Q D L L S E E N E S T A L P Q V L A Q P S T S R K 520 521 R P R E G E A E G A E T T K R P A V C A A V L </pre> (57) Abstract The invention provides for a novel human checkpoint kinase gene, hCDS1, translated protein, compositions, methods, and kits.		

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A NOVEL HUMAN CHECKPOINT KINASE, hCDS1, COMPOSITIONS AND METHODS

BACKGROUND OF THE INVENTION

The integrity of the genome is of prime importance to a dividing cell. In response to DNA damage, eukaryotic cells rely upon a complex system of checkpoint controls to delay cell-cycle progression. The normal eukaryotic cell-cycle is divided into 4 phases (sequentially G1, S, G2, M) which correlate with distinct cell morphology and biochemical activity, and cells withdrawn from the cell-cycle are said to be in G0, or non-cycling state. When cells within the cell-cycle are actively replicating, duplication of DNA occurs in the S phase, and active division of the cell occurs in M phase. *See generally* Benjamin Lewin, GENES VI (Oxford University Press, Oxford, GB, Chapter 36, 1997). DNA is organized in the eukaryotic cell into successively higher levels of organization that result in the formation of chromosomes. Non-sex chromosomes are normally present in pairs, and during cell division, the DNA of each chromosome replicates resulting in paired chromatids. (*See generally* Benjamin Lewin, GENES VI (Oxford University Press, Oxford, GB, Chapter 5, 1997).

Checkpoint delays provide time for repair of damaged DNA prior to its replication in S-phase and prior to segregation of chromatids in M-phase (Hartwell and Weinert, 1989, Science, 246: 629-634). In many cases the DNA-damage response pathways cause arrest by inhibiting the activity of the cyclin-dependent kinases (Elledge, 1997, Science, 274: 1664-1671). In human cells the DNA-damage induced G2 delay is largely dependent on inhibitory phosphorylation of Cdc2 (Blasina et al., 1997, Mol. Cell Biol., 8: 1-11; Jin et al., 1996, J. Cell Biol., 134: 963-970), and is therefore likely to result from a change in the activity of the opposing kinases and phosphatases that act on Cdc2. However, evidence that the activity of these enzymes is substantially altered in response to DNA damage is lacking (Poon et al., 1997, Cancer Res., 57: 5168-5178).

Three distinct Cdc25 proteins are expressed in human cells. Cdc25A is specifically required for the G1-S transition (Hoffmann et al., 1994, EMBO J., 13: 4302-4310; Jinno et al., 1994, EMBO J., 13: 1549-1556), whereas Cdc25B and Cdc25C are required for the G2-M transition (Gabrielli et al., 1996, J. Cell Sci., 7: 1081-1093; Galaktionov et al., 1991, Cell, 67: 1181-1194; Millar et al., 1991, Proc. Natl. Acad. Sci. USA, 88: 10500-10504; Nishijima et al., 1997, J. Cell Biol., 138:

1105-1116). The exact contribution of Cdc25B and Cdc25C to M-phase progression is not known.

Much of our current knowledge about checkpoint control has been obtained from studies using budding (*Saccharomyces cerevisiae*) and fission
5 (*Schizosaccharomyces pombe*) yeast. A number of reviews of our current understanding of cell cycle checkpoints in yeast and higher eukaryotes have recently been published (Hartwell & Kastan, 1994, Science, 266: 1821-1828; Murray, 1994, Current Biology, 6: 872-876; Elledge, 1996, Science, 274: 1664-1672; Kaufmann & Paules, 1996, FASEB J., 10: 238-247). In the fission yeast six gene products, *rad1**,
10 *rad3**, *rad9**, *rad17**, *rad26**, and *hus1** have been identified as components of both the DNA-damage dependent and DNA-replication dependent checkpoint pathways. In addition *cds1** has been identified as being required for the DNA-replication dependent checkpoint and *rad27**/*chk1** has been identified as required for the DNA-damage dependent checkpoint in yeast.

15 Several of these genes have structural homologues in the budding yeast and further conservation across eukaryotes has recently been suggested with the cloning of two human homologues of *S. pombe rad3**: ATM (ataxia telangiectasia mutated) (Savitsky *et al.*, 1995, Science, 268: 1749-1753) and ATR (ataxia telangiectasia and *rad3** related)(Bentley *et al.*, 1996, EMBO J., 15: 6641-6651; Cimprich *et al.*, 1996, Proc. Natl. Acad. Sci. USA, 93: 2850-2855) and of a human homologue of *S. pombe rad9** (Lieberman *et al.*, 1996, Proc. Natl. Acad. Sci. USA, 93: 13890-13885).
20

While much is known about yeast checkpoint proteins and genes, this knowledge is not fully predictive of the existence of corresponding human genes or proteins, or their effector role in human cell-cycle control and regulation.

25 In order to develop new and more effective treatments and therapeutics for the amelioration of the effects of cancer, it is important to identify and characterize human checkpoint proteins and to identify mediators of their activity.

SUMMARY OF THE INVENTION

The present invention is directed to the discovery of a novel human checkpoint
30 kinase gene hCDS1, protein and constructs and methods for the production and use of hCDS1.

In particular, the present invention encompasses a nucleic acid sequence which encodes for hCDS1, consisting of the nucleic acid sequence of SEQ ID NO.: 1. In

particular, the invention encompasses the nucleic acid sequence from position 66 to 1695 of the nucleic acid sequence of SEQ ID NO.: 1, which translates into the hCDS1 protein. The present invention also encompasses nucleic acid constructs, vectors, plasmids, cosmids and the like which contain the nucleic acid sequence of SEQ ID

5 NO.: 1. In particular, the present invention provides for nucleic acid vector constructs which contain the nucleic acid sequence of SEQ ID NO.: 1 and are capable of expressing protein from this nucleic acid sequence. The present invention encompasses nucleic acid vectors that are suitable for the transformation of host cells, whether eukaryotic or prokaryotic, suitable for incorporation into viral vectors, or suitable for

10 *in vitro* protein expression. The present invention further embodies the nucleic acid sequence of SEQ ID NO.: 1 in tandem with, or otherwise in conjunction with additional nucleic acids for the generation of fusion protein products containing at least the functional segment of the protein encoded for by the nucleic acid of SEQ ID NO.: 1. The present invention also encompasses the nucleic acid of SEQ ID NO.: 1

15 adapted for use as a naked DNA transformant for incorporation and expression in target cells. The present invention also provides for anti-sense DNA molecule formulations which are the complement to nucleic acid sequence of SEQ ID NO.: 1, and fragments thereof, whether complementary to contiguous or discontinuous portions of the nucleic acid sequence of SEQ ID NO.: 1. The present invention also

20 provides for compositions incorporating modified nucleotides or backbone components which encode for the nucleic acid sequence of SEQ ID NO.: 1, its complement, or fragments thereof. Such modified nucleotides and nucleic acids are known in the art (see for example Verma *et al.*, Ann. Rev. Biochem. 67: 99-134 (1998)). Thus the present invention encompasses modified nucleic acids which incorporate, for example,

25 internucleotide linkage modification, base modifications, sugar modification, nonradioactive labels, nucleic acid cross-linking, and altered backbones including PNAs (polypeptide nucleic acids).

The present invention provides for the novel human checkpoint kinase protein hCDS1, which consists of the amino acid sequence of SEQ ID NO.: 2. The invention

30 encompasses hCDS1 protein produced by recombinant DNA technology and expressed *in vivo* or *in vitro*. The invention thus encompasses hCDS1 protein produced by transformed host cells in small-scale or large-scale production. The invention encompasses complete hCDS1 protein, in either glycosylated or unglycosylated forms,

produced by either eukaryotic or prokaryotic cellr. The present invention provides for hCDS1 protein expressed from mammalian, insect, plant, bacterial, fungal, or any other suitable host cell. The present invention encompasses hCDS1 protein that is produced as a fusion protein product, conjugated to a solid support, or hCDS1 protein which is labeled with any chemical, radioactive, fluorescent, chemiluminescent or otherwise detectable marker. The present invention also provides for hCDS1 protein isolated from natural sources and enriched in purity over that found in nature. The present invention also provides for pharmaceutical formulations of hCDS1 protein and formulations of the hCDS1 protein in pharmaceutically acceptable carriers or excipients.

The present invention encompasses any nucleic acid sequence which would encode for the amino acid sequence of SEQ ID NO.: 2, and the embodiments of these nucleic acid sequences as described for SEQ ID NO.: 1, as the nucleic acid code for generating any nucleic acid sequence which will encode for a protein having the amino acid sequence of SEQ ID NO.: 2 is predictable to one of skill in the art.

The present invention encompasses antibodies which bind specifically to the hCDS1 protein, either polyclonal or monoclonal, as generated by the immunization of a mammal with protein having the amino acid sequence of SEQ ID NO.: 2, or fragments thereof.

The present invention also encompasses equivalent proteins where substitutions of amino acids in the sequence of SEQ ID NO.: 2 that are reasonably predictable as being equivalent, and the embodiments thereof as described for SEQ ID NO.: 2. For example, non-polar (hydrophobic side-chain) amino acids alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine; uncharged polar amino acids glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine; charged polar amino acids aspartic acid, glutamic acid; basic amino acids lysine, arginine, and histidine are understood by those in the art to have functionally predictable effects when substituted. Thus the present invention also encompasses equivalent nucleic acids which encode for such equivalent proteins and the embodiments thereof as described for SEQ ID NO.: 1.

The invention also provides for methods of generating hCDS1 protein, by using recombinant DNA technology and the appropriate nucleic acid encoding for hCDS1 protein, fusion protein, or fragments thereof. The invention provides for

incorporating an appropriate nucleic acid sequence into a suitable expression vector, the incorporation of any suitable control elements such as promoter, enhancer, either inducible or constitutively expressed. The invention provides for the use of expression vectors with or without at least one additional selectable marker or expressible protein.

5 The invention provides for methods wherein a suitably constructed expression vector is transformed or otherwise introduced into a suitable host cell, and protein is expressed by such a host cell. Thus the present invention also provides for the transformed host cells, which are capable of producing hCDS1 protein, fusion protein, or fragments thereof.

10 The discovery that hCDS1 acts in coordination with Cdc25 in the DNA damage checkpoint allows for the use of the compounds of the invention in methods for therapeutic treatment of diseases which involve abnormal DNA damage checkpoint function. The present invention further provides for the use of the compounds of the present invention as therapeutics for the treatment of cancer. In particular, the present
15 invention allows for the specific modification of the hCDS1-Cdc25 DNA damage checkpoint in cells.

The present invention also encompasses methods for screening test compounds for efficacy in effecting the hCDS1 mediated checkpoint function of eukaryotic cells, said method comprising contacting a test compound to eukaryotic cells, and detecting
20 any change in hCDS1 expression or function. Thus the invention further encompasses the method of screening wherein said detection of change in hCDS1 expression or function is accomplished by assaying for hCDS1 mRNA production, or by assaying for hCDS1 protein expression. In particular, the present invention allows for the screening of candidate substances for efficacy in modifying the DNA damage
25 checkpoint by screening for any change in Cdc25 phosphorylation, or kinase activity. The compounds or substances identified by the assays of the invention, or compounds corresponding to such compounds or substances, can be used for the manufacture of pharmaceutical therapeutics.

Thus, in one embodiment the present invention provides for pharmaceutical
30 compositions which include the hCDS1 protein, hCDS1 nucleic acid, hCDS1 anti-sense nucleic acids. In another embodiment, the present invention provides for compounds or substances identified as suitable for use as a therapeutic by the assays of the invention, in pharmaceutical formulations. These pharmaceutical compositions

can further include chemotherapeutic agents for the use in treating cancer, or be administered in a regimen coordinated with the administration of other anti-cancer therapies. The present invention, in one embodiment thus encompasses methods for combined chemotherapy using the hCDS1 derived pharmaceuticals independently, and
5 in combination with other chemotherapeutic agents, and in a second embodiment as admixtures with other anti-cancer therapeutics for single dose administration.

According to one aspect of the present invention, there is provided a nucleic acid encoding hCDS1 protein having the amino acid sequence illustrated in Figure 2 (SEQ ID NO.: 2), or encoding a functional equivalent fragment, or bioprecursor of
10 said protein. Preferably, the nucleic acid may be a DNA molecule such as a genomic DNA molecule and even more preferably a cDNA molecule, however it may also be RNA.

In a preferred embodiment, a nucleic acid encoding hCDS1 protein comprises the nucleic acid sequence represented by position 66 to 1694 of the sequence
15 illustrated in Figure 1 (SEQ ID NO.: 1), the complement thereof, or a nucleic acid sequence capable of hybridizing to either under high stringency conditions.

The nucleic acid sequences defined herein may, advantageously, be capable of hybridizing under low stringency conditions to nucleic acid sequences derived from family members to identify homologs therefrom or alternatively to identify nucleic
20 acid sequences from other species.

As would be well known to those skilled in the art due to the degeneracy of the genetic code the nucleic acid sequences according to the invention may include substitutions therein yet which still encode the same amino acid sequence.

Advantageously, the nucleic acids according to the invention may be
25 incorporated into an expression vector and may be subsequently used to transform, transfect or infect a suitable host cell. In such an expression vector the nucleic acid according to the invention is operably linked to a control sequence, such as a suitable promoter or the like, ensuring expression of the proteins according to the invention in a suitable host cell. The expression vector may, advantageously be a plasmid, cosmid,
30 virus or other suitable vector. The expression vector and the host cell transfected, transformed or infected with the vector also form part of the present invention. Preferably, the host cell is a eukaryotic cell or a bacterial cell and even more preferably a mammalian cell or insect cell. Mammalian host cells are particularly

advantageous because they provide the necessary post-translational modifications to the expressed proteins according to the invention, such as glycosylation or the like, which modifications confer optimal biological activity on said proteins, which when isolated may advantageously be used in diagnostic kits or the like.

5 The expression vector including said nucleic acid according to the invention may advantageously be used *in vivo*, such as in, for example, gene therapy.

 According to a further aspect of the invention there is also provided a transgenic cell, tissue or organism comprising a transgene capable of expressing hCDS1 protein, which protein comprises the amino acid sequence illustrated in Figure
10 2 (SEQ ID NO.: 2), or the amino acid sequence of a functional equivalent or bioprecursor or fragment therefor. The term "transgene capable of expression" as used herein means a suitable nucleic acid sequence which leads to expression of hCDS1 or proteins, having the same function and/or activity. The transgene may include, for example, genomic nucleic acid isolated from human cells or synthetic
15 nucleic acid, including DNA integrated into the genome or in an extrachromosomal state. Preferably, the transgene comprises the nucleic acid sequence encoding the proteins according to the invention as described herein, or a functional fragment of said nucleic acid. A functional fragment of said nucleic acid should be taken to mean a fragment of the gene comprising said nucleic acid coding for the proteins according
20 to the invention or a functional equivalent, derivative or a non-functional derivative such as a dominant negative mutant, or bioprecursor of said proteins. For example, it would be readily apparent to persons skilled in the art that nucleotide substitutions or deletions may be used using routine techniques, which do not affect the protein sequence encoded by said nucleic acid, or which encode a functional protein according
25 to the invention.

 The hCDS1 protein expressed by said transgenic cell, tissue or organism or a functional equivalent or bioprecursor of said protein also form part of the present invention.

 Further provided by the present invention is an antisense molecule which is
30 capable of hybridizing to the nucleic acid according to the invention. Advantageously, the antisense molecule according to the invention may be used as a medicament, or in the preparation of a medicament for the treatment of cancer and other proliferative diseases.

The present invention also advantageously provides nucleic acid sequences of at least approximately 15 nucleotides of a nucleic acid according to the invention and preferably from 15 to 50 nucleotides. These sequences may advantageously be used as probes or primers to initiate replication, or the like. Such nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention. These tests generally comprise contacting the probe with the sample under hybridizing conditions and detecting for the presence of any duplex or triplex formation between the probe and any nucleic acid in the sample.

Advantageously, the nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be from approximately 15 to 50 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region (and where necessary first performing a reverse transcription step), isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques as defined herein are well known in the art, such as described in Sambrook *et. al.*, (Molecular Cloning; a Laboratory Manual, 1989). Advantageously, human allelic variants of the nucleic acid according to the invention may be obtained by for example, probing genomic DNA libraries from a range of individuals for example from different populations, and other genotyping techniques. Furthermore, nucleic acids and probes according to the invention may be used to sequence genomic DNA from patients, using techniques well known in the art, for example, the Sanger dideoxy chain termination method, which may advantageously ascertain any predisposition of a patient to certain proliferative disorders.

Further provided by the present invention are isolated proteins having the amino acid sequences as illustrated in Figure 2 (SEQ ID NO.: 2) or the amino acid sequence of a functional equivalent functional fragment or bioprecursor of said protein in addition to antibodies, monoclonal or polyclonal capable of binding to the amino acid sequences of these proteins or fragments thereof. As would be well known to

those skilled in the art, the proteins according to the invention may comprise conservative substitutions, deletions or insertions wherein the protein comprises different amino acids than those disclosed in Figure 2, yet which substitutions, deletions or insertions do not affect the activity of the proteins according to the invention or their ability to interact in the human cell cycle checkpoint pathway.

Preferred fragments include those comprising an epitope of the proteins according to the invention. The epitopes may be determined using, for example, peptide scanning techniques as described in Geysen *et. al.*, Mol. Immunol., 23; 709-715 (1986).

The antibodies according to the invention may be produced according to techniques which are known to those skilled in the art. Monoclonal antibodies may be prepared using conventional hybridoma technology as described in Kohler F and Milstein C (1985), Nature 256, 495-497. Polyclonal antibodies may also be prepared using conventional technology well known to those skilled in the art, and which comprises inoculating a host animal, such as a mouse, with a protein or epitope according to the invention and recovering the immune serum. The present invention also includes fragments of whole antibodies which maintain their binding activity, such as for example, Fv, F(ab') and F(ab')₂ fragments as well as single chain antibodies.

Advantageously, the nucleic acid and/or the proteins according to the invention may be included in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor. The pharmaceutical composition containing said nucleic acids according to the invention may, for example, be used in gene therapy. Such nucleic acids, according to the invention, may be administered naked, or packaged in protein capsules, lipid capsules, liposomes, membrane based capsules, virus protein, whole virus, cell vectors, bacterial cell hosts, altered mammalian cell hosts, or such suitable means for administration.

There is further provided by the present invention a method for detecting for the presence or absence of a nucleic acid according to the invention, in a biological sample, which method comprises, a) bringing said sample into contact with a probe comprising a nucleic acid or probe according to the invention under hybridizing conditions, and b) detecting for the presence of hybridization, for example, by the presence of any duplex or triplex formation between said probe and any nucleic acid

present in said sample. Proteins according to the invention may also be detected by a) contacting said sample with an antibody to an epitope of a protein according to the invention under conditions which allow for the formation of an antibody-antigen complex, b) monitoring for the presence of any antigen-antibody complex.

5 Kits for detecting said nucleic acids and proteins are also provided by the present invention. A kit for detecting for the presence of a nucleic acid according to the invention in a biological sample may comprise (a) means for contacting the sample with a probe comprising a nucleic acid or a probe according to the invention and means for detecting for the presence of any duplex or triplex formation between
10 said probe and any nucleic acid present in the sample.

 Likewise, a kit for detecting for the presence of a protein according to the invention in a biological sample may comprise (a) means for contacting said sample with an antibody to an epitope of a protein according to the invention under conditions which allow for the formation of an antibody - protein complex, and means
15 for monitoring said sample for the presence of any protein - antibody complex.

 A further aspect of the present invention provides a method of determining whether a compound is an inhibitor or an activator of expression or activity of the proteins of the human cell cycle checkpoint pathway which method comprises contacting a cell expressing the proteins in said pathway with said compound and
20 comparing the level of expression of any of the proteins of the checkpoint pathway of said cell against a cell which has not been contacted with said compound. Any compounds identified may then advantageously be used as a medicament or in the preparation of a medicament for treating cancer or proliferative disorders.

 Alternatively, the compounds may be included in a pharmaceutical composition together with a pharmaceutically acceptable carrier, diluent or excipient therefor.
25 Advantageously, any compounds identified as an inhibitor of the cell checkpoint pathway may be included in a pharmaceutical composition according to the invention together with a cytotoxic agent, such as a DNA damaging chemotherapeutic agent, and a pharmaceutically acceptable carrier diluent or excipient therefor. Thus, the human
30 cell cycle checkpoint inhibitor may enhance the chemotherapeutic effect of cytotoxic agents used in, for example, anti-cancer therapy.

 There is also provided by the present invention a method for screening candidate substances for anti-cancer therapy, which method comprises a) providing a

protein according to the present invention exhibiting kinase activity together with a substrate for said protein under conditions such that the kinase will act upon the substrate, b) bringing the protein and substrate into contact with a candidate substance, c) measuring the degree of any increase or decrease in the kinase activity of the protein, d) selecting a candidate substance which provides a decrease or increase in activity. Such a candidate substance may also be used as a medicament, or in the preparation of a medicament for the treatment of cancer or other such proliferative cell disorders.

The present invention also comprises a method of identifying other proteins active in the cell checkpoint pathway, which method comprises a) contacting a cell extract with an antibody to an epitope of a protein according to the invention, under appropriate binding conditions, b) identifying any antibody-protein complex and c) analyzing the complex to identify any protein bound to the antibody or protein which is other than the protein according to the invention.

Another method for identifying proteins involved in the cell checkpoint pathway utilizes a two-hybrid system developed in yeast by Chien *et. al.*, *supra* (1991). This technique is based on functional in vivo reconstitution of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or the activating domain of the transcription factor, expressing in the host cell at least one second hybrid DNA sequence encoding putative binding proteins to be investigated together with the DNA binding domain or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the protein being investigated with a protein according to the invention by detecting for the production of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein. In one embodiment of this aspect of the invention the method may comprise:

(a) constructing at least two nucleotide vectors, the first of which comprises a nucleotide segment encoding for a DNA binding domain of GAL4 protein operably

linked to a nucleic acid sequence encoding a protein according to the present invention, the second vector comprising a nucleotide sequence encoding a protein binding domain of GAL4 operably linked to a nucleotide sequence encoding a protein to be tested,

- 5 (b) co-transforming each of said vectors into a yeast cell being deficient for transcription of genes encoding galactose metabolizing proteins, wherein interaction between said test protein and the protein according to the invention leads to transcription of galactose metabolic genes.

BRIEF DESCRIPTION OF THE DRAWINGS

- 10 The invention may be more clearly understood from the following examples which are given by way of example only, with reference to the accompanying drawings, wherein:

FIGURE 1 illustrates the nucleotide sequence of hCDS1 (SEQ ID NO.: 1) wherein residues 66-1694 is the coding region and 3' and 5' untranslated regions(UTRs). The initiation and termination codons are shown in bold,

15 FIGURE 2 illustrates the deduced amino acid sequence of hCDS1 (SEQ ID NO.: 2),

FIGURE 3 illustrates the amino acid sequence alignment of hCDS1 and *S. pombe cds1* performed using the CLUSTALW alignment program and annotated using the GENEDOC program. Residues shaded black are identical between the two proteins and residues shaded grey are similar.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention encompasses the isolation and characterization of a novel human checkpoint kinase gene and protein which is called hCDS1. The hCDS1 gene and protein show some similarity to a homologous gene and protein found in *S. pombe*.

30 The *S. pombe cds1*⁺ gene was identified by its ability to complement a DNA polymerase α mutant (Murakami & Okayama, 1995, Nature, 374: 817-819). *S. pombe cds1* was also able to suppress the hydroxyurea sensitivity (DNA replication-dependent checkpoint) of *rad1*, *rad3* and *rad9* mutant *S. pombe* strains but not the UV sensitivity (DNA damage-dependent checkpoint). This shows that *S. pombe cds1* executes its checkpoint function during DNA synthesis.

S. pombe cds1 is a putative protein kinase that is 70% similar to the *S. cerevisiae* checkpoint gene RAD53. In *S. cerevisiae* the DNA damage- and DNA replication-dependent checkpoints are genetically separate at the level of detection of DNA lesions. The two pathways then converge on the Rad53 protein kinase which potentially acts as an amplifier in the signal transduction pathway. This appears not to be the case in *S. pombe* where the same proteins are involved in detection of all types of lesion but the transduction of the signal follows separate pathways involving different protein kinases; *S. pombe* cds1 for the DNA replication-dependent checkpoint and Chk1/Rad27 for the DNA damage-dependent pathway. It has been suggested that S-phase-specific activation of cds1 kinase may define a subpathway of the checkpoint response in *S. pombe* (Lindsay et al., 1998, Genes and Development, 12: 382-395).

S. pombe cds1 may act via an interaction with DNA polymerase α to monitor the progress of DNA replication or the integrity of replication complexes. There is evidence in *Drosophila* for a kinase of the appropriate molecular weight associating with DNA polymerase α (Peck et al., 1993, B.B.R.C., 190: 325-331). Alternatively it may act via phosphorylation of p107^{wee1} in a manner analogous to Chk1 ultimately affecting the activity of the G1/S phase cyclin dependent kinases.

Many of the methods and materials for carrying out the basic molecular biology manipulations as described in the examples below are known in the art, and can be found in such references as Sambrook et al., Molecular Cloning, 2nd edition, Cold Spring Harbor Laboratory Press (1989); Berger et al., Guide to Molecular Cloning Techniques, *Methods in Enzymology*, Vol. 152, Academic Press, Inc., (1987); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing Co., Inc. (1986); Ausubel et al., Short Protocols in Molecular Biology, 2nd ed., John Wiley & Sons, (1992); Goeddel Gene Expression Technology, *Methods in Enzymology*, Vol. 185, Academic Press, Inc., (1991); Guthrie et al., Guide to Yeast Genetics and Molecular Biology, *Methods in Enzymology*, Vol. 194, Academic Press, Inc., (1991); McPherson et al., PCR Volume 1, Oxford University Press, (1991).

The invention in its several aspects can be more readily understood by reviewing the following examples.

Example 1 Isolation of hCDS1

Isolation of hCDS1 began with a search for sequences similar to *S. pombe cds1+* using the TBLASTN program. A human expressed sequence tag (EST No. 864164) was identified in the proprietary LifeSeq^(R) database (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA). Sequence analysis of the 1.3 kb insert revealed an
5 incomplete open reading frame which was similar to *S. pombe cds1*. Approximately 650 nucleotides of novel 5' DNA sequence was obtained by 5'RACE (rapid amplification of cDNA ends) using a Marathon Ready human placental cDNA (Clontech), following the manufacturer's instructions.

10 Briefly, the two hCDS1 gene specific primers used for nested PCR (Polymerase chain reaction) reactions were GSP3 5'-TTTGTGCTGATGATCTTTATGGCTAC-3' (SEQ ID NO.: 3) and GSP4 5'-CACAGGCACCACTTCCAAGAGTTTT-3' (SEQ ID NO.: 4). Subsequently, a complete ORF for hCDS1 was amplified from a human SK-N-MC neuroblastoma
15 cDNA library using the PCR primers 5'-GGGCTCGAGAGCAGCGATGTCTCGGGAGTCGGATGT-3' (SEQ ID NO.: 5) and 5'-GGCGGATCCTCGAGTCACAACACAGCAGCACACAC-3' (SEQ ID NO.: 6). The amplification product was then cloned into pCR2.1 vector (Invitrogen) and the
20 DNA sequence determined.

The nucleic acid sequence of hCDS1 was found to show 47.8% identity to the *S. pombe cds1+* at the DNA level. Termination codons were present in all three reading frames in the 120 nucleotides immediately 5' to the putative hCDS1 initiation codon, indicating that the complete coding region has been isolated. Parts of the
25 sequence is also found to match partial sequences found in the NCBI databases, EST AA285249, genomic sequence H55451, and the 54 base pair fragment H55698.

The identified human gene and vectors encoding the hCDS1 nucleic acid sequence were deposited as plasmid HCDS1 ORF/pCR-Blunt deposited under Accession No. LMBP 3708; plasmid HCDS1 5'RACE fragment/pGEM-Easy deposited
30 under Accession No. LMBP 3710; and plasmid HCDS1 3'fragment Incyte clone 864164/pSPORT deposited under Accession No. LMBP 3709 with the Belgian Co-ordinated Collections of Micro-organisms (BCCM) at Laboratorium Voor Moleculaire

Biologies-Plasmidencollecte (LMBP) 35, B-9000 Gent, Belgium, in accordance with the provisions of the Budapest Treaty, 28 April 1997.

The tissue expression profile of hCDS1 was examined on multiple tissue Northern blots (Clontech) and a cancer cell line Northern blot (Clontech), which were probed with the hCDS1 ORF. A single transcript of approximately 2.1 kb was observed. Expression was undetectable by conventional Northern blot hybridization conditions in all normal human tissues examined. However, expression was found to be greatly elevated in all of the cancer cell lines examined.

The hCDS1 gene was localized to chromosome 22q11.2-q12, as determined using the complete ORF as a probe for FISH (Fluorescent *in situ* Hybridization) analysis. The hybridization efficiency was approximately 62%, and no other loci were detected under the conditions used.

Briefly, lymphocytes isolated from human blood were cultured in α -minimal essential media (MEM) supplemented with 10% fetal calf serum and phytohaemagglutinin (PHA) at 37°C for 68-72 hours. The lymphocyte cultures were treated with BrdU (0.18 mg/ml, Sigma) to synchronize the cell population. The synchronized cells were washed three times with serum-free medium to release the block and re-cultured at 37°C for 6 hours in α -MEM with thymidine (2.5 μ g/ml Sigma). Cells were harvested and slides were prepared using standard procedures including hypotonic treatment, fixation and air-drying. DNA fragments containing the hCDS1 complete ORF were gel purified and biotinylated with dATP using the BRL BioNick labeling kit (15°C, 1 hour) (Heng et al., 1992, Proc. Natl. Acad. Sci. USA. 89: 9509-9513).

Slides were then baked at 55°C for 1 hour, and after RNase treatment, the slides were denatured in 70% formamide in 2x SSC for 2 minutes at 70°C followed by dehydration with ethanol. Probes were denatured at 75°C for 5 minutes in a hybridization mix consisting of 50% formamide and 10% dextran sulphate. Probes were loaded on the denatured chromosomal slides. After overnight hybridization, slides were washed and detected. FISH signals and the DAPI-banding pattern were recorded separately by taking photographs, and the assignment of the FISH mapping data with chromosomal bands was achieved by superimposing FISH signals with DAPI banded chromosomes (Heng & Tsui, 1994, Methods in Mol. Biol., 33: 35-49).

Example 2 Characterization of hCDS1 protein

The hCDS1 nucleic acid sequence cDNA predicts a translation product of 543 amino acids with an approximate molecular weight of 61kDa. This is close to the apparent molecular weight of endogenous Cds1 protein in HeLa cells. The predicted hCDS1 protein, is 28% identical to the cds1 protein of *S. pombe*, 28% identical to RAD53 and 27% identical to the DUN1 kinase of *S. cerevisiae*. Sequence alignment of these apparent homologs shows several regions of sequence similarity outside the kinase domain, including conservation of the Fork Head Associated domain (Hoffmann et al., 1995, Trends Biochem. Sci., 20: 347-9). The human protein shows the same overall structure as *S. pombe* CDS1 and *S. cerevisiae* DUN1 in that it lacks the long C-terminal extension found in RAD53. Northern blot analysis with hCDS1 identified a single transcript of about 2.2 kb expressed in testis and in 8 human cancer samples examined.

Briefly, two multiple tissue Northern blots (Clontech) and a Cancer Cell line Northern blot (Clontech) were hybridized with a cDNA probe for hCDS1. The probe corresponds to the complete ORF as described above. The blots were washed at high stringency (0.1 x SSC, 0.1% SDS, 50°C, 2 x 20 min) and exposed using Kodak X-OMAT autoradiography film with intensifying screens at -70°C.

Example 3 Cdc25 total activity assay

The possibility that dephosphorylation of Cdc2 is down-regulated in the presence of DNA damage required an assay to allow for the analysis of the total activity of Cdc25. In the presence of EDTA, Cdc2/Cyclin B from asynchronous HeLa cell extracts was found to inactivate spontaneously.

Briefly, cells were lysed in ice-cold lysis buffer (50 mM Tris pH 7.4 containing 2 mM magnesium chloride, 1 mM phenylmethylsulphonyl fluoride, and 5 µg/ml leupeptin, pepstatin and aprotinin). Lysates were cleared by centrifugation at 10,000 xg for 10 minutes and the protein concentration of the supernatants determined using the Lowry assay. 10 mM EDTA was added to the supernatants (100 µg in 60 µL) and the reaction initiated by incubation at 30°C. At assay intervals the activity of Cdc2/Cyclin B was assayed by measuring the histone-H1 kinase activity present in anti-Cyclin B immune-precipitates (Blasina et al., *supra.*). For immunoblots 400 µg of cell lysate was immune-precipitated using anti-Cyclin B antibody, resolved on an 11%

acrylamide-SDS gel. Monoclonal antibody against the PSTAIRE motif of Cdc2 was used to detect the different phospho-forms of Cdc2.

Activation correlates with loss of the inhibited-phosphorylated form of Cdc2, visualized as the slower migrating species on SDS-PAGE gels. Activation was prevented by vanadate, an inhibitor of Cdc25 and other tyrosine phosphatase. Furthermore, immune-depletion with Cdc25C-specific anti-sera greatly reduced activation of Cdc2/Cyclin B. There was no increase in the levels of Cdc2 or Cyclin B protein, phosphorylation by WEE1 and Myt1 was blocked by the presence of 10 mM EDTA. Thus, these results demonstrate that the activation of Cdc2 was the result of dephosphorylation. In lysates of asynchronous HeLa cells, the endogenous Cdc25 phosphatase activity is sufficient to dephosphorylate and activate more than 80% of the available Cyclin B/Cdc2 in 30 minutes. Analysis of lysates of HeLa cells in which the DNA had been damaged by exposure to 10 Gy of γ -irradiation one hour before harvesting showed a significant reduction in the rate of activation of Cdc2, such that less than 25% of the available Cdc2/Cyclin B was activated during the 30 minutes incubation. The amount of Cdc2/Cyclin B in complex was not significantly altered and it was activated to the same extent as control Cdc2/Cyclin B by addition of exogenous GST-Cdc25. Irradiation with 10 Gy led to more than 3-fold reduction in the rate of Cdc2 dephosphorylation in the 10 time courses examined. If the inactivation of Cdc25 measured above is part of the DNA-damage checkpoint response in human cells, then experimental conditions that over-ride the DNA damage checkpoint might be expected to block the radiation-induced inhibition of Cdc25.

Example 4 DNA Damage Checkpoint Effect of hCDS1

DNA damage response in a variety of cells is known to require various related kinases which structurally are related to PI-3 kinases. At least one member of the family, DNA-Protein Kinase, has been shown to be sensitive to wortmannin *in vitro* (Hawley et al., 1996, Genes and Dev., 10: 2383-8; Hartley et al., 1995, Cell, 82: 849-856). Thus the possibility that a wortmannin-sensitive kinase acted upstream of the radiation induced delay in M-phase entry was tested (Price et al., 1996, Cancer Research, 56: 246-250). HeLa cells can be arrested in M-phase by nocodazole, irradiation causes cells to delay in G2 prior to the nocodazole-sensitive M-phase block point. Thus, by scoring the mitotic index of cells that are cultured in nocodazole, it is

possible to determine whether entry into mitosis has been delayed. Control cells cultured in the presence of nocodazole for 14 hours contained 60% mitotic cells, the presence of wortmannin had little effect on this number. However, irradiation reduced the number of cells that reach the nocodazole block point to only 10%. In contrast, irradiation in the presence of wortmannin had only a modest effect. These results demonstrate that wortmannin over-rides the DNA damage G2 checkpoint in HeLa cells.

The effects of wortmannin on the radiation-induced inactivation of Cdc25 was then tested. Wortmannin had little effect on the activation of Cdc2/Cyclin B in extracts prepared from unirradiated cultures, however it did greatly diminish the irradiation-induced decrease in Cdc25 activity.

Radiation-induced G2 checkpoint is also over-ridden in cell-lines derived from patients with the genetic disorder ataxia telangiectasia. Ataxia Telangiectasia mutant cells (ATM) are defective in both the G1 and G2 checkpoints following exposure to many, but not all, agents that damage DNA (Canman et al., 1994, Cancer Research, 54: 5054-5058). The failure of AT-deficient cells to delay G1 correlates with a failure to up-regulate p53 (Kastan et al., 1992, Cell, 71: 587-589), and with failure to phosphorylate and activate cAb1 (Baskaran et al., 1997, Nature, 387: 516-519; Shafman et al., 1997, Nature, 387: 520-523). The molecular basis for the failure to delay G2 is unknown. AT-deficient cells show greatly reduced responses to agents that generate chromosomal breaks, such as ionizing γ -rays. Remarkably, AT-deficient cells have near normal responses following the base damage that is generated by irradiation with a UV source (Canman et al., 1994, Cancer Research, 54: 5054-5058; Painter et al., 1980, Proc. Natl. Acad. Sci. USA, 77: 7315-7317; Zampetti-Bosseler et al., 1981, Int. J. Radiat. Biol., 39: 547-558). The effects of UV and γ -irradiation on the Cdc25 activity of AT-plus and AT-minus SV40-transformed human fibroblast cell-lines was tested. AT-minus cells respond to UV-irradiation with a robust reduction in the rate at which Cdc2 is dephosphorylated. In contrast, γ -irradiation had only a modest effect on the rate of dephosphorylation of Cdc2. In AT-plus cells the rate of dephosphorylation of Cdc2 was significantly reduced following either ionizing-radiation or UV-radiation. These data indicate that the ATM gene product is required for the efficient inactivation of Cdc25 following γ -irradiation and demonstrate a correlation between inactivation of Cdc25 and delayed entry into M-phase following DNA damage.

Mediators of the checkpoint-dependent inactivation of Cdc25 in human cells are excellent targets for generating therapeutics or therapeutic regimens that will enhance anti-cancer treatment, and reduce side-effects on normal cells.

To facilitate biochemical characterization of hCDS1, 6his-hCDS1 was
5 expressed in insect cells, affinity purified and incubated in extracts of HeLa cells in the presence of an ATP-regenerating system. EDTA was added to inhibit kinase in the extract, and the rate of dephosphorylation and activation of Cdc2/CyclinB was monitored.

Briefly, recombinant viruses encoding for 6his-hCDS1, 6his-Chk1, 6his-Cdc2
10 and GST-Cdc25C were generated using the Bac-to-Bac expression system from Gibco/BRL. 6his-fusion proteins were purified following the procedure described in Kumagai et al., (1995), Mol. Biol. Cell, 6: 199-213. GSH-sepharose beads were incubated for 15 minutes in Sf9 extracts; beads were collected by centrifugation and washed three-times with lysis buffer (50 mM Tris pH 8.0, 5 mM EDTA, 150 mM
15 NaCl, 0.1% NP40, 5% glycerol, 0.1% β -mercaptoethanol and protease inhibitors). Beads were washed three-times with kinase assay buffer (50 mM Tris pH 7.4, 10 mM $MgCl_2$) prior to phosphorylation reactions or three-times with phosphatase assay buffer (50 mM imidazole pH 7.4, 5 mM EDTA and 0.1% β -mercaptoethanol) prior to phosphatase assays.

Both 6his-Chk1 and 6his-hCDS1 were found to significantly reduce the
20 activation of Cdc2/Cyclin B in these assays. The reduced activation of Cdc2 was dose dependent and required ATP. Confirmation that Cdc2 was not irreversibly inhibited by 6his-Chk1 or 6his-hCDS1 was shown by the activation that resulted when excess GST-Cdc25C was added after kinase treatment. Thus, both 6his-hCDS1 and 6his-Chk1
25 can mimic the radiation-induced down-regulation of Cdc25 seen in extracts. These experiments used HeLa cell lysates that had been clarified by centrifugation, therefore it is unlikely that changes in sub-cellular locale could account for inactivation of Cdc25 (Peng et al., 1997, Science, 277: 1501-1505).

30 Example 5 Direct Effect of hCDS1 on Cdc25

Indirect mechanisms of inhibition of Cdc25 by hCDS1 could not be excluded by the cell lysate assays, therefore, affinity-purified reagents were used to determine direct phosphorylation and inhibition of GST-Cdc25 activity by hCDS1.

GST-Cdc25 was incubated with either 6his-hCDS1, mock beads, or 6his-Chk1 in the presence of γ -32P ATP for 15 minutes at 30°C. Proteins were resolved by SDS-PAGE and visualized by autoradiography. GST-Cdc25 was phosphorylated by 6his-Chk1 and by 6his-hCDS1. Assays were performed to determine if Cdc25 phosphatase activity was effected by this phosphorylation.

GST-Cdc25 was assayed for its ability to activate the histone-H1 kinase activity of Cdc2/Cyclin B immune-precipitates. It was found that phosphorylation of GST-Cdc25 by 6his-hCDS1 inhibited the ability of GST-Cdc25 to activate Cdc2/Cyclin B. Thus, these data demonstrate that 6his-hCDS1 inactivated Cdc25 *in vitro*, and that Cdc25 is inactivated *in vivo* following DNA damage.

Since 6his-Chk1 associates with GST-Cdc25 and has histone-H1 kinase activity *in vitro* (Sanchez et al., 1997, Science, 277: 1497-1501), analysis of Cdc2/Cyclin B kinase activity was obscured. In order to test GST-Chk1 effects, an assay was used in which Cdc2 dephosphorylation was monitored by the disappearance of the slower migrating species of Cdc2 on gel-mobility analysis.

Briefly, phosphorylated Cdc2 was purified from Sf9 cells that had been simultaneously infected with recombinant baculoviruses encoding 6his-Cdc2, 6his-Wee1, 6his-Myt1 and GST-Cyclin B (Parker et al., 1992, Science, 257: 1955-1957). The 6his-Cdc2 complexed to Cyclin B was purified using GSH beads under the conditions for GST-Cdc25 except that 1 mM VO_4 was included in the lysis buffer. Western Blot analysis showed that quadruple infection resulted in phosphorylation of the majority of Cdc2/GST-Cyclin B at one or both inhibitory sites. These phosphatase assays were carried out in the presence of 10 mM EDTA, and the absence of ATP, conditions that eliminate the possibility of 6his-Chk1 phosphorylating Cdc2 or Cyclin B directly. GST-Cdc25 catalyses a reduction in the slower migrating phosphorylated forms of Cdc2. Prior phosphorylation of GST-Cdc25 by 6his-Chk1 leads to a dose-dependent reduction in GST-Cdc25 activity. These data confirm that Chk1 negatively regulated Cdc25 activity (Furnari et al., 1997, Science, 277: 1495-1497; Weinert, 1997, Science, 277: 1450), and extend them by demonstrating that the negative regulation involves inactivation of the phosphatase activity.

Example 6 DNA Damage and Modification of hCDS1

As the previous data had shown that 6his-hCDS1 inactivates Cdc25, and that DNA damage is associated with Cdc25 inactivation, an assay was performed to determine if DNA damage leads to any modification or activation of hCDS1. Antisera raised against 6his-hCDS1 was used in immune-complex kinase assays using HeLa cell lysates. A weak signal corresponding to hCDS1 was detected in the sample from asynchronous HeLa cells; increased phosphorylation of hCDS1 was seen following irradiation.

Briefly, antibodies to hCDS1 were generated by immunizing a rabbit with 6his-hCDS1 purified from Sf9 cells (Harlow et al., Antibodies (Cold Spring Harbor Laboratory Press, NY, 1988). The resulting antisera immune-precipitates an active kinase of the expected molecular weight from Sf9 cells infected with 6his-hCDS1 virus, but not from uninfected Sf9 cells, or from other cells infected with 6his-Chk1 virus.

The results were confirmed as being due to hCDS1 by re-precipitation of the protein band following denaturation in 4% SDS. The *in vitro* phosphorylation is most likely due to autophosphorylation, and the increased signal reflects an increase in activity following irradiation. The increase *in vitro* phosphorylation of p64^{Cds1} suggests that, like RAD53 and DUN1, hCDS1 is modified in response to DNA damage.

The effect of arresting DNA synthesis on phosphorylation of p64^{Cds1} was examined by further assay. The hCDS1 from replication arrested cells behaved exactly like the protein from asynchronous cultures; no significant increase in phosphorylation was seen in response to thymidine or other agents that block DNA replication. The increased phosphorylation of p64^{Cds1} was detected following irradiation of thymidine-arrested cells. The effect of damaging DNA in cells that are predominantly arrested outside S-phase was also tested. Cells were cultured in the presence of nocodazole for 20 hours prior to irradiation. Again, a weak but detectable signal was seen in the unirradiated sample. However, irradiation of nocodazole arrested cells lead to increased phosphorylation.

These findings surprisingly contrast with the results found in yeast, where fission yeast Cds1 has been found to be activated in response to incompletely replicated DNA (Boddy et al., 1998, Science, 280: 909-12; Lindsay et al., 1998, Genes and Dev., 12: 382-95). The results here show a role for human Cds1 in the

DNA damage checkpoint rather than the replication checkpoint as previously found in yeast.

Example 7 Drug Identification

5 The Cdc25 assays described above are suitable for use in the identification of chemical agents that would modify the DNA damage checkpoint mediated by hCDS1 and Cdc25, either by enhanced or inhibited activity. Thus a typical screening assay would use similar conditions as described above, plus addition of a reagent to be tested. Monitoring of the activity of the assay components, i.e. detection of
10 phosphorylation as described above, can be conducted in comparison to control reactions to detect both enhanced and inhibited activity.

 Clearly such assays are readily adaptable to mechanical/automated apparatus and detection. With the fundamental elements of the assay reactions being known, the assay is clearly suited for use in conjunction with automated high-throughput
15 low-signal apparatus which may incorporate microscopic slide array, or cell-biochip arrays in conjunction with CCD detection devices and the use of a visible signal triggered by phosphorylation or other reaction to kinase activity.

Example 8 Therapeutic Use

20 The characterization of hCDS1 and the elucidation that the role for human Cds1 is in the DNA damage checkpoint rather than the replication checkpoint as found in yeast, allows for the adaptation of this knowledge to the preparation of pharmaceuticals, and therapeutic methods for acting as an adjunct to chemotherapy of cancer.

25 In particular, pharmaceutical formulations of the present invention incorporating cDNA, RNA, antisense molecules, hCDS1 protein, antibodies against hCDS1 protein, or other therapeutics corresponding to those identified in the assays of the invention, can be administered in conjunction with any suitable chemotherapy agent in order to act as an adjunct to the main action of the chemotherapy agent. For
30 example, the use of anticancer drugs such as antimetabolite, antibiotics, alkylating agents, microtubule inhibitors, steroid hormones and their antagonists, and others, is generally directed against metabolic sites essential to cell replication. While ideally these drugs should intervene only with the cellular processes unique to malignant

cells, the currently available anticancer drugs affect all proliferating cells, both normal and malignant. Thus, current chemotherapy is hampered by a steep dose-response curve for both toxic and therapeutic effects. Therefore, co-administration of the hCDS1-based drugs of the present invention, and drugs identified by the hCDS1
5 assays of the present invention, with chemotherapeutic agents will allow for enhanced killing of malignant cells.

One mechanism for enhanced killing is effected by disabling the DNA damage checkpoint control of malignant cells, thus making the administration of DNA
10 damaging chemotherapeutic agents more effective. The disabling of the DNA damage control checkpoint can be effected by modifying the hCDS1 response, as demonstrated by the data above.

Thus, the co-administration of novel hCDS1 based therapeutics in combination with any one or more anticancer agent is contemplated by the present invention. For example, normal dosages of the anticancer drugs Cytarabine, Fludarabine,
15 5-Fluorouracil, 6-Mercaptopurine, Methotrexate, 6-Thioguanine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Idarubicin, Plicamycin, Carmustine, Iomustine, Cyclophosphamide, Ifosfamide, Mechloroethamine, Streptozotocin, Navelbine, Paclitaxel, Vinblastine, Vincristine, Asparaginase, Cisplatin, Carboplatin, Etoposide, Interferons, Procarbazine etc., can be administered with the appropriate
20 amount of hCDS1 based drug so as to a) alter the length of time of administration, b) alter the time between administrations, c) alter the efficacy of the chemotherapeutic agent on malignant cells, or d) alter the side-effects of the chemotherapeutic agent on normal cells. The effects of the co-administration of hCDS1 based drugs can be any one or combination of these effects in addition to others.

25 Typically, destruction of cancer cells by chemotherapeutic agents follows first-order kinetics, for a log kill effect. Thus, the co-administration of hCDS1-based therapeutics would be designed to enhance the log kill effect. Typically, chemotherapeutic treatment protocols call for a combination of drugs which act at different steps in the metabolic pathway, thus enhancing killing while staying below
30 toxic levels. Thus, the co-administration of hCDS1 based therapeutics would ideally be in combination with such protocols, and improve efficacy thereof.

Ultimately, the most effective therapeutic methods would combine targeted administration of chemotherapeutic drugs and/or MDR (multidrug resistance)

inhibiting agents, with hCDS1 based therapeutics, to specifically target and eliminate malignant cells via the cells' own uncontrolled replication without DNA damage repair, and thus eventual cell death.

5 The foregoing discussion and examples are intended as illustrative of the present invention and are not to be taken as limiting. Still other variants within the spirit and scope of this invention are possible and will readily present themselves to those of skill in the art.

What is claimed is:

1. An isolated nucleic acid encoding for HCDS1, having the nucleic acid sequence represented from position 66 to 1694 of the nucleic acid sequence of Figure 1 (SEQ ID NO.: 1).

2. An isolated nucleic acid encoding for a protein having the amino acid sequence of Figure 2 (SEQ ID NO.: 2), or encoding a functional equivalent, fragment or bioprecursor of such protein.

3. A nucleic acid according to claim 2 which is a DNA molecule.

4. A nucleic acid according to claim 3 wherein said DNA molecule is a cDNA.

5. A nucleic acid according to any of claims 3 or 4, having the nucleic acid sequence represented from position 66 to 1694 of the nucleic acid sequence of Figure 1 (SEQ ID NO.: 1), or the complement thereof or a sequence capable of hybridizing thereto under high stringency conditions or the complement thereto.

6. An antisense molecule comprising a nucleic acid which is capable of hybridizing to the nucleic acid of any of claims 1 to 5, under high stringency conditions.

7. A nucleic acid according to any of claims 1 to 6 wherein said nucleic acid contains a modification selected from the group consisting of, internucleotide linkage modification, nucleotide base modification, nucleotide sugar modification, nonradioactive labeling, nucleic acid cross-linking and peptide nucleic acid modification.

8. A nucleic acid according to any of claims 1 to 7 for use in treating cancer or other proliferative disease.

9. A nucleic acid according to any of claims 1 to 7 for use in the manufacture of a medicament.

10. The use of a nucleic acid according to any of claims 1 to 7 for use in the manufacture of a medicament.

11. A pharmaceutical composition comprising a nucleic acid according to claims 1 to 7 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

12. The use of a pharmaceutical composition comprising a nucleic acid according to claims 1 to 7 together with a pharmaceutically acceptable carrier, diluent or excipient therefor, for the treatment of cancer or other proliferative disease.

13. The method of treating cancer or proliferative disease, said method comprising the step of administering a nucleic acid according to claims 1 to 7 to a patient in need of treatment.

5 14. The method of treating cancer or proliferative disease, said method comprising the step of administering a pharmaceutical composition comprising a nucleic acid according to claims 1 to 7 together with a pharmaceutically acceptable carrier, diluent or excipient therefor, to a patient in need of treatment.

15. A nucleic acid probe which comprises a fragment of at least 15 nucleotides of the nucleic acid as defined by any of claims 1 to 7.

10 16. A method of detecting for the presence or absence of a nucleic acid of claim 1 in a biological sample, which method comprises, bringing said sample into contact with a probe comprising a nucleic acid according to any of claims 1 to 7, or a probe according to claim 15 under hybridizing conditions, and detecting for the presence of any duplex or triplex formation between said probe and any nucleic acid
15 present in said sample.

17. An isolated plasmid having the identifying characteristics of LMBP 3708.

18. An isolated plasmid having the identifying characteristics of LMBP 3710.

19. An isolated plasmid having the identifying characteristics of LMBP 3709.

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FIG. 1

HCDS1 cDNA nucleotide sequence

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1   actagtgatt actcacaggg ctcgagcggc cgcccgggca_ggtcaggtgg 50
51  gctcacgcgg tcgtgatgtc tcgggagtcg gatgttgagg ctcacagtc 100
101 tcatggcagc agtgccctgtt cacagcccca tggcagcgtt acccagtc 150
151 aaggctcctc ctcacagtc ccagggcata ccagctcctc taccagcacg 200
201 atgccaaact ccagccagtc ctctcactcc agctctggga cactgagctc 250
251 cttagagaca gtgtccactc aggaactcta ttctattcct gaggaccaag 300
301 aacctgagga ccaagaacct gaggagccta ccctgcccc ctgggctcga 350
351 ttatgggccc ttcaggatgg atttgccaat cttgaatgtg tgaatgacaa 400
401 ctactggttt gggaggggaca aaagctgtga atattgcttt gatgaaccac 450
451 tgctgaaaag aacagataaa taccgaacat acagcaagaa acactttcgg 500
501 attttcaggg aagtgggtcc taaaaactct tacattgcat acatagaaga 550
551 tcacagtggc aatggaaacct ttgtaaatac agagcttgta gggaaaggaa 600
601 aacgccgtcc tttgaataac aattctgaaa ttgactgtc actaagcaga 650
651 aataaagttt ttgtcttttt tgatctgact gtagatgac agtcagttta 700
701 tcctaaggca ttaagagatg aatacatcat gtcaaaaact cttggaagtg 750
751 gtgcctgtgg agaggtaaag ctggctttcg agaggaaaac atgtaagaaa 800
801 gtagccataa agatcatcag caaaaggaag ttgctattg gttcagcaag 850
851 agaggcagac ccagctctca atgttgaaac agaaatagaa attttgaaaa 900
901 agctaaatca tccttgcatc atcaagatta aaaacttttt tgatgcagaa 950
951 gattattata ttgttttgga attgatggaa gggggagagc tgtttgacaa 1000
1001 agtggtgagg aataaacgcc tgaaagaagc tacctgcaag ctctattttt 1050
1051 accagatgct cttggctgtg cagtaccttc atgaaaacgg tattatacac 1100
1101 cgtgacttaa agccagagaa tgttttactg tcatctcaag aagaggactg 1150
1151 tcttataaag attactgatt ttgggcactc caagattttg ggagagacct 1200
1201 ctctcatgag aaccttatgt ggaaccccca cctacttggc gcctgaagtt 1250
1251 cttgtttctg ttgggactgc tgggtataac cgtgctgtgg actgctggag 1300
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1851 gaaaaaaa

```

2/3

FIG. 2

Predicted hCDS1 Amino acid sequence

1 MSRES DVEAQ QSHGSSACSQ PHGSVTQSQG SSSQSQGISS 40
41 SSTSTMPNSS QSSHSSSGTL SSLETVSTQE LYSIPEDQEP 80
81 EDQEPEEPTP APWARLWALQ DGFANLECVN DNYWFGRDKS 120
121 CEYCFDEPLL KRTDKYRTYS KKHFRIFREV GPKNSYIAYI 160
161 EDHSGNGTFV NTELVGKGKR RPLNNNSEIA LSLSRNKV FV 200
201 FFDLTVDDQS VYPKALRDEY IMSKTLGSGA CGEVKLA FER 240
241 KTCKKVAIKI ISKRKFAIGS AREADPALNV ETEIEILKKL 280
281 NHPCIIKIKN FFDAEDYYIV LELMEGGELF DKVVVG NKRLK 320
321 EATCKLYFYQ MLLAVQYLHE NGIIHRDLKP ENVLLSSQEE 360
361 DCLIKITDFG HSKILGETSL MRTLCGTPTY LAPEVLVSVG 400
401 TAGYNRAVDC WSLGVILFIC LSGYPPFSEH RTQVSLKDQI 440
441 TSGKYNFIPE VWA EVSEKAL DLVKKLLVVD PKARFTTEEA 480
481 LRHPWLQDED MKRK FQDLLS EENESTALPQ VLAQPSTSRK 520
521 RPREGEAEGA ETTKRPAVCA AVL

3/3

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HCDS1      : MPNSSQSSSHSSSGTLLSSLETVSTQELYSHPEDQEPEDQEPPEPTAPPWARLWALQDGFANLECVNDNYM-FGRDKSCEY : 78
S.p.CDS1    : ME---EPEEATQATQEAPLHVSQNTAKQVNN-----ENVFMKLVMTRMLDGKTEVIPLTDDVHNGFTGFGGRHKSCEV : 70

HCDS1      : CFDEPLLKRRTDKYRTYSKKHFRIFREVGPKNSYIAVIEDHSSNGTFTVMTLVCKGKRRPLANNSEIALSLSRNK----- : 152
S.p.CDS1    : VLAGF---RVSNFHFEIYQCHRNDSDES--EN--VVEIHDHSSNGTFTVMTLVCKGKRRPLANNSEIALSLSRNKDEISF : 143

HCDS1      : -VFVFFDLTVDDQSVYPKALRDEYIMSKTLGSGACGEVKLAFERKTCCKVAIKIISKRKFATCSAREADPALNVFTEIE : 230
S.p.CDS1    : LCQVPVKHSRDSCKNMIKSENSHYEIIRTLGSCTFAVVKLAVEVNSCKWYAIKIINRRKILLTSS-EKRATEMFOREID : 221

HCDS1      : ILRKENHPCTIIKIKNFFDAED-YIVIVLEIMEGGELFDKWKGNKRKEATCKLYFYQMLLAQYLHENGIIHRDLKPENV : 308
S.p.CDS1    : ILKSTHHPGVVQCHEICENDDELEFIWMEYVEGGDLMDFLIANGSIDQCKPLLKQLLETLLHLEKQGVTHRDIKPENI : 300

HCDS1      : LLSQEEEDCLIKITDFGHSKILGET-SLARILCGTPTTYLAPEVLVSVGT---AGYNRAVDCMSLGVILFTCLSGYPPFS : 383
S.p.CDS1    : LITN----CFHLKISDFGLAKVVIHGTGTFLEIFCGTGMGYLAPEVLKSKNVNLDGGYDDKJDIISLGCCLYVYMLTASIPFA : 376

HCDS1      : EHRQVSLKDOITSGKNFIPVWAEVSEKALDLVKKLLVDPKARFTTEALRHPALQDEDMKRKFQDLLSEENESTA : 462
S.p.CDS1    : SS-SQAKCTELISKGAVPIEPLIENEISEEGIDLINRMLEINPEKRISESEALQHPIFYT----- : 435

HCDS1      : LPQVLAQPSTSEKRPREGGAEAGAEFTTKRPVCAAVL : 498
S.p.CDS1    : ----VSTHEHRTPESSSEHEATEQLNSSS----- : 460

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FIG. 3

SEQUENCE LISTING

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 and Methods
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 Ser Ala Cys Ser Gln Pro His Gly Ser Val Thr Gln Ser Gln Gly Ser
 20 25 30
 tcc tca cag tcc cag ggc ata tcc agc tcc tct acc agc acg atg cca 206
 Ser Ser Gln Ser Gln Gly Ile Ser Ser Ser Thr Ser Thr Met Pro
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 Asn Ser Ser Gln Ser Ser His Ser Ser Ser Gly Thr Leu Ser Ser Leu
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 gag aca gtg tcc act cag gaa ctc tat tct att cct gag gac caa gaa 302
 Glu Thr Val Ser Thr Gln Glu Leu Tyr Ser Ile Pro Glu Asp Gln Glu
 65 70 75
 cct gag gac caa gaa cct gag gag cct acc cct gcc ccc tgg gct cga 350
 Pro Glu Asp Gln Glu Pro Glu Glu Pro Thr Pro Ala Pro Trp Ala Arg
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 Leu Trp Ala Leu Gln Asp Gly Phe Ala Asn Leu Glu Cys Val Asn Asp
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 Asn Tyr Trp Phe Gly Arg Asp Lys Ser Cys Glu Tyr Cys Phe Asp Glu
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 Lys Thr Leu Gly Ser Gly Ala Cys Gly Glu Val Lys Leu Ala Phe Glu
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Thr Glu Ile Glu Ile Leu Lys Lys Leu Asn His Pro Cys Ile Ile Lys
att aaa aac ttt ttt gat gca gaa gat tat tat att gtt ttg gaa ttg 974
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Gln Tyr Leu His Glu Asn Gly Ile Ile His Arg Asp Leu Lys Pro Glu
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 Gln Ser Val Tyr Pro Lys Ala Leu Arg Asp Glu Tyr Ile Met Ser Lys
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 Thr Leu Gly Ser Gly Ala Cys Gly Glu Val Lys Leu Ala Phe Glu Arg
 225 230 235 240
 Lys Thr Cys Lys Lys Val Ala Ile Lys Ile Ile Ser Lys Arg Lys Phe
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 Ala Ile Gly Ser Ala Arg Glu Ala Asp Pro Ala Leu Asn Val Glu Thr
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 Glu Ile Glu Ile Leu Lys Lys Leu Asn His Pro Cys Ile Ile Lys Ile
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 Lys Asn Phe Phe Asp Ala Glu Asp Tyr Tyr Ile Val Leu Glu Leu Met
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 Glu Gly Gly Glu Leu Phe Asp Lys Val Val Gly Asn Lys Arg Leu Lys
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 Glu Ala Thr Cys Lys Leu Tyr Phe Tyr Gln Met Leu Leu Ala Val Gln
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 Tyr Leu His Glu Asn Gly Ile Ile His Arg Asp Leu Lys Pro Glu Asn
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 Val Leu Leu Ser Ser Gln Glu Glu Asp Cys Leu Ile Lys Ile Thr Asp
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 Phe Gly His Ser Lys Ile Leu Gly Glu Thr Ser Leu Met Arg Thr Leu
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 Cys Gly Thr Pro Thr Tyr Leu Ala Pro Glu Val Leu Val Ser Val Gly
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/54, 9/12, A61K 48/00, C12Q 1/68		A3	(11) International Publication Number: WO 99/20747
			(43) International Publication Date: 29 April 1999 (29.04.99)
(21) International Application Number: PCT/EP98/06982 (22) International Filing Date: 21 October 1998 (21.10.98) (30) Priority Data: 9722320.0 22 October 1997 (22.10.97) GB (71) Applicant (for all designated States except US): JANSSEN PHARMACEUTICA N.V. [BE/BE]; Turnhoutseweg 30, B-2340 Beerse (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): LUYTEN, Walter, H., M., L. [BE/BE]; Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse (BE). PARKER, Andrew, E. [GB/GB]; Zeneca Pharmaceuticals, 8AF22 Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). (74) Agents: LEAPER, Lyn; Janssen Pharmaceutica, Turnhoutseweg 30, B-2340 Beerse (BE) et al.		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 1 July 1999 (01.07.99)	
(54) Title: HUMAN CHECKPOINT KINASE, hCDS1, COMPOSITIONS AND METHODS <div style="text-align: center;"> Predicted hCDS1 Amino acid sequence </div> <pre> 1 MSRES DVEAQ QSHGSSACSQ PHGSVTQSQG SSSQSQGISS 40 41 SSTSTMPNSS QSSHSSSGTL SSLETVSTQE LYSIPEDQEP 80 81 EDQEPEEPTP APWARLWALQ DGFANLECVN DNYWFGDRDKS 120 121 CEYCFDEPLL KRTDKYRTYS KKHFRIFREV GPKNSYIAYI 160 161 EDHSGNGTFV NTELVGKGKR RPLNNNSEIA LSLSRNKVVFV 200 201 FFDLTVDDQS VYPKALRDEY IMSKTLGSGA CGEVKLA FER 240 241 KTCKKVAIKI ISKRKFAIGS AREADPALNV ETEI EILKKL 280 281 NHPCIIKIKN FFDAEDYYIV LELMEGGELF DKVVG NKRLK 320 321 EATCKLYFYQ MLLAVQYLHE NGIIHRDLKP ENVLLSSQEE 360 361 DCLIKITDFG HSKILGETSL MRTLCGTPTY LAPEVLVSVG 400 401 TAGYNRAVDC WSLGVILFIC LSGYPPFSEH RTQVSLKDQI 440 441 TSGKYNFIPE VWA EVSEKAL DLVKKLLVVD PKARFTTEEA 480 481 LRHPWLQDED MKRKFQDLLS EENESTALPQ VLAQPSTSRK 520 521 RPREGEAEGA ETTKRPAVCA AVL </pre>			
(57) Abstract The invention provides for a novel human checkpoint kinase gene, hCDS1, translated protein, compositions, methods, and kits.			

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/06982

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/54 C12N9/12 A61K48/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLAUDIO ET AL.: "Identification of sequence-tagged transcripts differentially expressed within the human hematopoietic hierarchy" EMBL DATABASE, 9 April 1997, XP002100553 Heidelberg AC AA285249 see the whole document & GENOMICS, vol. 50, no. 1, 1998, pages 44-52, ---	5,6,15
A	WO 96 28555 A (UNIV JEFFERSON ;GIORDANO ANTONIO (US)) 19 September 1996 see abstract; claims 7,20-22 see page 3, line 10 - page 4, line 8 see page 4, line 25 - line 33 see page 5, line 32 - page 6, line 9 --- -/--	1-19

☒

Further documents are listed in the continuation of box C.

☒

Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

23 April 1999

Date of mailing of the international search report

10/05/1999

Name and mailing address of the ISA

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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Ceder, O

INTERNATIONAL SEARCH REPORT

Int. J. Application No.

PCT/EP 98/06982

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CIMPRICH K A ET AL: "CDNA CLONING AND GENE MAPPING OF A CONDIDATE HUMAN CELL CYCLE"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, April 1996, pages 2850-2855, XP002023632 cited in the application see abstract</p>	1-4
A	<p>SANCHEZ Y ET AL: "Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25 'see comments!'"</p> <p>SCIENCE, vol. 277, no. 5331, 5 September 1997, pages 1497-1501, XP002087723 cited in the application see page 1498</p>	1,2,16
A	<p>WO 97 32473 A (UNIV WASHINGTON) 12 September 1997 see page 3, line 12 - line 21; claim 17</p>	1,8, 12-14,16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 98/06982

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 13 and 14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/06982

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